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Biological Activity of Feijoa Peel Extracts

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Abstract. *Fractionated extracts of Feijoa peels were studied for cytotoxic activity, anti-human immunodeficiency virus (HIV) activity and antibacterial activity. Two most cytotoxic fractions A3 of acetone extract and M2 of methanol extract had potent inhibitory activity against Gram-positive and Gram-negative bacteria as well as fungi tested. Fraction A4 of acetone extract showed multidrug resistance (MDR)-reversal activity comparable with that of verapamil (positive control). These results indicate the therapeutic value of Feijoa peel extracts as potential antimicrobial and MDR-modulating agents.*

Feijoa sellowiana Berg (Myrtaceae) has been cultured mainly in the tropics and subtropics such as south Brazil, Uruguay, Paraguay and North Argentina. Feijoa has a pleasant flavour and is also eaten stewed, in jams, jelly or juice. Feijoa contains many medicinally bioactive compounds.

The predominant aroma of Feijoa fruits is ascribed to methylbenzoate and ethylbenzoate (1, 2). Other volatile components were also identified (3-7). Feijoa is rich in vitamin P (P)-active polyphenols, such as catechin, leucoanthocyanins, flavonols, proanthocyanidins and naphthoquinones (8-11). The Feijoa leaves of also contain catechins such as (+)-catechin, (-)-epicatechin, (+)-gallocatechin and (-)-epigallocatechin (12). Furthermore, tannins in Feijoa fruits and leaves have been identified (13). The flower part of Feijoa contains anthocyanin-3-glucoside of polyphenols (14). Feijoa fruits are rich in vitamin C (8, 10, 15-17). Especially, a concentrated juice of Feijoa has 3-times higher concentrations of vitamin C than fresh juice (5). Provitamin A such as α-carotene, β-carotene and β-cryptoxanthin were also determined (18). Both water-insoluble and water-soluble fibers are found in Feijoa (17, 19). Amino acids such as tryptophan, lysine, methionine and asparagine with nutritional value were also extracted from Feijoa (17, 20). A recent study on the biological activities of an aqueous extract from Feijoa fruit has shown both antibacterial and antioxidant properties (21).

In addition, interestingly, Feijoa peel contains especially high amounts of vitamin C and catechin-like vitamin P (P)-active polyphenols, such as leucoanthocyanins, flavonols and naphthoquinones (15). Although the chemical constituents of the Feijoa fruit have been reported, no detailed pharmaceutical study of the constituents of Feijoa peel has as yet been performed.

The peel is generally regarded as a waste matter although, biologically and nutritiously interesting compounds are rich in it. With this consideration in mind, we have screened for new biologically active agents from various fruits and vegetables that are consumed by people. The purpose of this paper was to study the cytotoxic activity, anti-human immunodeficiency virus (HIV) activity and antibacterial activity of Feijoa peel extracts.

Materials and Methods

Materials. Feijoa sellowiana Berg was collected from Motohashi garden in Tokyo. Voucher specimens of the plants were deposited at the herbarium of Josai University.

Preparation of Feijoa peel extracts. Feijoa peel (330 g) was cut into small pieces and successively extracted with hexane, acetone, MeOH and 70% MeOH at room temperature; the solvent was concentrated *in vacuo* and the hexane extract [**H0**](0.44 g), acetone extract [**A0**](5.9 g), MeOH extract [**M0**](16.7 g) and 70% MeOH extract [**70M0**](6.1 g) were obtained, respectively. First, the aliquot of hexane extract

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[**H0**](0.4 g) was applied to silica gel column chromatography, which was then eluted with a hexane-acetone gradient. The hexane fraction [**H1**](32 mg), hexane-acetone (9:1) fraction [**H2**](157 mg), hexane-acetone (4:1) fraction [**H3**](47 mg) and [**H4**](45 mg) were eluted stepwise. Second, the acetone extract [**A0**](5.5 g) was applied to silica gel column chromatography, which was then eluted with a benzene-AcOEt gradient. The benzene fraction [**A1**](92 mg), benzene-AcOEt (10:1) fraction [**A2**](103 mg), [**A3**](145 mg), benzene-AcOEt (1:1) fraction [**A4**](170 mg), AcOEt fraction[**A5**](582 mg), AcOEt-EtOH (5:1) fraction [**A6**](836 mg) and [**A7**](854 mg) were eluted stepwise. Third, the MeOH extract [**M0**](16.7 g) was applied to silica gel column chromatography, which was then eluted with a CH_2Cl_2-MeOH gradient. The CH_2Cl_2 fraction [**M1**](24 mg), CH_2Cl_2-MeOH (50:1) fraction [**M2**](135 mg), CH_2Cl_2-MeOH (9:1) fraction [**M3**](25 mg), [**M4**](761 mg), CH_2Cl_2-MeOH (4:1) fraction [**M5**](1317 mg) and [**M6**](2100 mg) were eluted stepwise. Finally, the 70% MeOH extract [**70M0**](5 g) was applied to ODS column chromatography, which was then eluted with a H_2O-MeOH gradient. The H_2O-MeOH (2:1) fraction [**70M1**](2010 mg), [**70M2**](80 mg), H_2O-MeOH (1:1) fraction [**70M3**](18 mg), [**70M4**](43 mg) and MeOH fraction [**70M5**](1200 mg) were eluted stepwise (Figure 1).

Assay for cytotoxic activity. Human oral squamous cell carcinoma (HSC-2) and human oral salivary gland tumor (HSG) cell lines and human oral gingival fibroblasts (HGF) (5-7 population doubling levels) were cultured in Dulbecco's modified Eagle medium (DMEM) (Gibco BRL, Grand Island, NY, U.S.A.) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (JRH Biosci). These cells were incubated for 24 hours with the indicated concentrations of test samples and the viable cell number was then determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In brief, the cells were washed with phosphate-buffered saline (PBS) and incubated for 4 hours with fresh culture medium containing 0.2 mg/mL MTT (Sigma Chem. Ind., St. Louis, MO, U.S.A.). After removing the medium, the cells were lysed with 100 μL DMSO and the absorbance at 540 nm of the cell lysate was measured with Labsystems Mutiskan^R (Biochromatic) with Star/DOT Matrix printer JL-10. The 50% cytotoxic concentration (CC_{50}) was determined from the dose-response curve.

Assay for anti-HIV activity. The inhibition of HIV-induced cytopathic effects by Feijoa extracts was studied. Human T cell leukemia virus 1 (HTLV1)-bearing CD4 positive human T cell lines, MT-4 cells, were infected with HIV-1_{IIIB} at a multiplicity of infection (m.o.i.) of 0.01. HIV- or mock-infected MT-4 cells (1.5×10^5/mL, 200 μL) were placed into 96-well microtiter plates in RPMI 1640 medium supplemented with 10% heat-inactivated PBS and incubated in the presence of varying concentrations of the compounds tested. After incubation for 5 days at 37°C in a CO_2 incubator, cell viability was quantified by a colorimetric assay (at 540 nm and 690 nm), monitoring the ability of viable cells to reduce MTT to a blue formazan product (22). All data represent the mean values of triplicate measurements.

Bacterials. Escherichia coli LE 140 F'lac, *Pseudomonas aeruginosa, Staphylococcus epidermidis, Candida albicans* and *Candida glabrata* were isolated from clinical speciments obtained from the Department of Clinical Microbiology, Medical University of Szeged, Hungary. *Helicobacter pylori* (ATCC43504) was purchased from the American Type Culture Collection (Rockville, MD, U.S.A.).

Measurement of antibacterial activity. The experiments were made by adding 10 μL of original solutions as a droplet on a minimal medium supplemented with 1% trypton and 0.5% yeast extract (MTE) broth and blood agar plates inoculated with 10^5 cells of the tested strains. The plates were incubated at 37°C for 24 hours, then the inhibitory zones were measured. As a control, 10 μL of DMSO was examined on each strain. It was found that *C. albicans, C. glabrata* and *P. aeruginosa* were sensitive to DMSO. The growth of *E. coli* and *S. epidermidis* was not inhibited by DMSO. An ampicillin disc was used as a positive control in the studies of antibacterial effects.

Measurement of anti-H. pylori activity. The micro-dilution broth method was used to determine the minimum inhibitory concentration (MIC). Mueller-Hilton broth containing 5% FBS was used as the medium and was cultured in a jar conditioned with Campylo Pack (Dia Iatron) for 48 hours. Briefly, *H. pylori* strains were inoculated on a Brucella agar plate containing 10% horse serum and cultured at 37°C for 48 hours. The collected bacterial colonies were diluted to 10^7 colony forming unit (UFU)/mL with 0.9% saline. The fractions were dissolved in DMSO and then diluted with Mueller-Hilton broth. To the solution of the fractions, a suspension of bacteria was added to make 10^6 CFU/100 mL/well. The mixture was incubated at 37°C for 48 hours. The MIC values of the fractions tested were determined by observation.

Cell and fluorescence uptake. MRD1/A expressing cell lines were selected by culturing the infected cells with 60 ng/mL colchicine to maintain the expression of the multidrug resistance (MDR) phenotype. The L5178 MDR cell line and the L5178 Y parent cell line were grown in McCoy's 5A medium supplemented with 10% heat-inactivated horse serum, L-glutamine and antibiotics. The cells were adjusted to a density of 2×10^6/mL and resuspended in serum-free McCoy's 5A medium and an 0.5 mL aliquot of the cell suspension were distributed into each Eppendorf centrifuge tube. Then, 2 μL of 2 mg/mL each test fraction was added and incubated for 10 min at room temperature. Then, 50 μL rhodamine 123 (R123) (5.2 μM final concentration) was added as indicator and the cells were incubated for a further 20 minutes at 37°C. The cells were washed twice and resuspended in 0.5 mL phosphate-buffered saline (PBS) for the analysis of the fluorescence of cell population by flow cytometry (Beckton Dickinson FACScan). Verapamil was used as the positive control in R123 accumulation experiments. R123 accumulation was measured by the fluorescence of the cells in argon laser. Then, the mean fluorescence intensity of the drug-treated cells was measured in parental and MDR cell lines, compared to untreated cells. The fluorescence activity ratio was calculated by using the following equation (23, 24):

Ratio = (MDR treated / MDR control) / (parental treated / parental control)

Results and Discussion

Cytotoxic activity. It is widely accepted that in cancer cells, many anticancer agents act as apoptosis-inducers and the major reason for the unresponsiveness of cancer cells is the insufficiency of these drugs to trigger apoptosis (25). The cytotoxicity of all fractions was evaluated in two human oral tumor cell lines (HSG-2, HSG) and one human gingival fibroblast (HGF), using the microculture tetrazolium (MTT) assay. Crude hexane [**H0**], acetone [**A0**], MeOH [**M0**] and 70%MeOH [**70M0**] extracts showed weak cytotoxic activity against these cells. However, after separation by column chromatography (Figure 1), various levels of cytotoxic activities became detectable in several fractions (Table I). Most fractions exhibited only low cytotoxicity (IC_{50} values>100 μg/mL,). However, fractions **A3** and **M2** displayed the highest cytotoxic activity against both tumor cell lines (HSC-2 and HSG) and the normal

Table I. *Cytotoxic and anti-HIV activity of Feijoa peel extracts against tumor and normal cells.*

Fraction	Cytotoxic activity (CC_{50}, μg/mL)				Anti-HIV activity		
	HSC-2	HSG	HGF	SI (HGF/HSC-2)	CC_{50} (μg/mL)	EC_{50} (μg/mL)	SI (CC_{50}/EC_{50})
HO	381	386	395	1.0	100	>200	<1
H1	>500	>500	>500	>>1	>200	>200	>>1
H2	313	357	370	1.2	162	>200	<1
H3	>500	>500	>500	>>1	>200	>200	>>1
H4	269	356	409	1.5	117	>200	<1
AO	444	462	452	1.0	119	>200	<1
A1	160	302	305	1.0	121	>200	<1
A2	132	123	165	1.3	113	>200	<1
A3	81	79	80	1.0	22	>40	<1
A4	274	318	382	1.4	46	>200	<1
A5	335	383	403	1.2	127	>200	<1
A6	>500	>500	>500	>>1	>200	>200	>>1
A7	>500	>500	>500	>>1	>200	>200	>>1
MO	>500	>500	>500	>>1	>200	>200	>>1
M1	378	427	441	1.2	>200	>200	>>1
M2	119	101	152	1.3	120	>200	<1
M3	338	268	277	0.8	78	>200	<1
M4	>500	458	235	<0.5	>200	>200	>>1
M5	>500	>500	>500	>>1	>200	>200	>>1
M6	458	>500	>500	>>1.1	>200	>200	>>1
70MO	380	>500	>500	>1.3	>200	>200	>>1
70M1	396	>500	>500	>1.3	>200	>200	>>1
70M2	394	>500	>500	>1.3	99	15	7
70M3	250	>500	>500	>2	83	>200	<1
70M4	208	280	419	2.0	102	>200	<1
70M5	488	>500	>500	>1.0	>200	>200	>>1
CRDS					>1000	1.1614	861
DS					>1000	13.9969	>71
AZT(μM)					268	0.3402	787
ddC (μM)					2395	6.6587	360

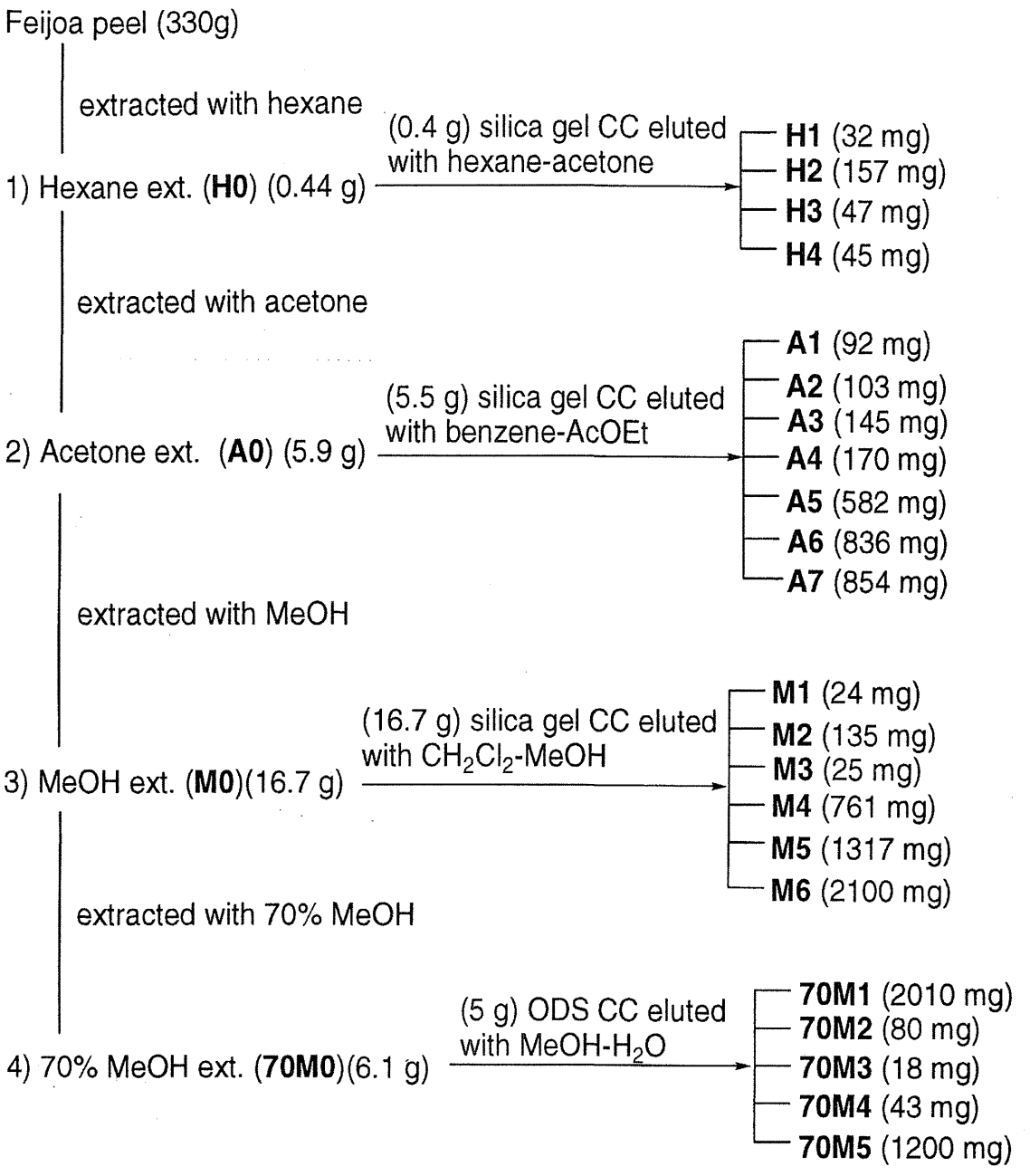

Figure 1. *Fractional separation of Feijoa peel extracts. CC: column chromatography.*

fibroblast (HGF). When the water solubility of the test fraction was increased, the relative cytotoxic activity against normal cells (SI=HGF/HSC-2) generally declined (Table I).

Anti-HIV activity. Table I shows that most of the fractions failed to inhibit HIV-induced cytopathic effects on MT-4 cells (selectivity index (SI)=1), compared to the higher anti-HIV activities of curdlan sulfate (CRDS), dextran sulfate (DS), AZT and ddC (SI=71-861). However, fraction **70M2,** which has relatively higher water-solubility, showed some anti-HIV activity (SI=7) (Table I). This is consistent with our previous findings that the anti-HIV activity increased with water-solubility of the compounds: in the order of lignin (26) > hydrolyzable tannins > condensed tannins (22). Higher molecular weight lignins and tannins showed higher anti-HIV activity in comparison with their lower molecular weight compounds (22, 26).

Table II. *Antibacterial activity of Feijoa peel extracts.*

Fraction	*Staphylococcus epidermidis*	*E. coli*	*Pseudomonas aeruginosa*	*Candida albicans*	*Candida glabrata*	*Helicobacter pylori* (MIC50, μg/mL)
H0	-	-		-		ND[b]
H1	-	-		-		>100
H2	+	+		+		>100
H3	-	+		-		>100
H4	-	+		-		>100
A0	-	-		-		ND[b]
A1	-	+-	-	++	-	>100
A2	-	++	+	++	-	>100
A3	++	++	+	++	+	>100
A4	-[a]	-		-		>100
A5	-	++		-		>100
A6	-	-		-		>100
A7	-	-		-		>100
M0	-	-		-		ND[b]
M1	-[a]	-	+	+	-	>100
M2	+	++	+	++	-	>100
M3	-[a]	+-	+	+	+	>100
M4	-	-		-		>100
M5	-	-		-		>100
M6	-	-		-		>100
70M0	-	+		-		ND[b]
70M1	-	-		-		>100
70M2	-[a]	+		-		>100
70M3	-[a]	+		-		>100
70M4	-[a]	+		-		>100
70M5	-	-		-		>100
Metronidazole						74
Clarithromycin						1.9
Erythromycin						1.8

++ very potent; + potent; +- slightly potent; - no effect.
[a] inhibited haemolysis.
[b] Not detected.

Antibacterial activity. Chemotherapy with antibiotics sometimes possesses serious side effects such as diarrhea, nausea, abnormal taste, dyspepsia, abdominal pain/discomfort, headache and angioedema. Therefore, there is a strong demand for the predominance of beneficial effects of antibacterial agents over their side effects. In this sense the antibacterial activity of fruit or vegetable extracts may be superior as compared to many antibiotics. All fractions were tested against a panel of microorganisms, including the Gram-positive bacteria, *Staphylococcus epidermidis* and Gram-negative bacteria, *Escherichia coli.* and *Pseudomonas aeruginosa* and the fungi, *Candida albicans* and *Candida glabrata* (Table II). The highest activities consistently resided in the acetone and MeOH extract fractions. Two fractions [**A3**] and [**M2**] were found to exhibit potent activity against both Gram-positive and -negative bacteria as well as against fungi.

Anti-Helicobacter pylori activity. The current medical consensus shows that *H. pylori* is the primary causative agent of acute gastritis (27). Since the drug will directly come in contact with the lining of the stomach, edible plants may be a good source of oral antiulcer agents. However, all fractions did not exhibit potent anti-*H. pylori* activity (MIC_{50}>100 μg/mL), in contrast to three effective positive controls: metronidazole, clarithromycin and erythromycin (Table II).

MDR reversal on tumor cells. Emerging drug resistance is known among viruses, bacteria, protozoa and cancer cells including retroviruses such as the HIV. The development of potent MDR inhibitors devoid of other toxicities has become a desirable goal to test the MDR-reversal hypothesis in the clinic.

Rhodamine 123 assay has been widely accepted as a direct and reproducible assay for measuring P-gycoprotein (Pgp)-dependent efflux (28). We measured the ability of the fractions to inhibit Pgp-mediated R-123 efflux in the MDR mouse T cell lymphoma L5178 transfected by human MDR 1 gene which was cultured in colchicine containing medium (Table III). Fraction **A4** had the highest MDR reversal activity (ratio: 19.90) among all fractions and was 1.3 times more potent than that of verapamil (ratio: 16.16), followed by fractions [**H3** (ratio: 4.55), **H4** (ratio: 5.38), **A5** (ratio: 5.07) and **70M4** (ratio: 9.34)]. It is clear that fraction **A4** was superior to all other fractions of Feijoa.

In conclusion, Feijoa peel extract has various bioactive components: Fractions **A3** and **M2** were not only cytotoxic, but also showed promising antibacterial activity. Fraction **A4** showed exceptionally promising levels of MDR-modulating activity. The results of this study apparently indicate the therapeutic value of Feijoa peel extracts. Further work is required to identify and isolate the compounds responsible for the reported biological activity.

Table III. *Effect of Feijoa peel extracts on the multi-drug resistance of L-5178 cells.*

Fraction	Concentration (μg/mL)	FSC[a]	SSC[a]	FL-1[a]	Fluorescence activity ratio
Par (control) [b]	10	394	131	4104.0	78.77
MDR+R123 (control)[c]	10	377	140	63.2	1.21
MDR+R123 (control)	10	448	143	41.0	0.79
MDR+R123 (mean)	10	413	142	52.1	1.00
(dl)-Verapamil	10	415	110	842.0	16.16
H0	20	331	363	821	3.09
H1	20	301	298	251	0.94
H2	20	317	227	311	1.17
H3	20	296	300	1206	4.55
H4	20	293	331	1419	5.35
A0	20	306	141	90	1.82
A1	20	377	173	190	3.86
A2	20	362	156	183	3.72
A3	20	332	152	165	3.30
A4	20	395	349	980	19.90
A5	20	305	168	249	5.07
A6	20	353	141	74	1.49
A7	20	379	141	74	1.49
M0	20	336	245	131	0.49
M1	20	345	236	188	0.71
M2	20	273	204	207	0.78
M3	20	311	233	807	3.04
M4	20	254	273	260	0.98
M5	20	266	236	109	0.41
M6	20	311	273	199	0.74
70M0	20	506	113	95	0.91
70M1	20	461	113	80	0.77
70M2	20	470	124	78	0.75
70M3	20	420	107	86	0.83
70M4	20	485	135	973	9.36
70M5	20	416	110	61	0.59

[a] FSC: Forward scatter count; SSC: Side scatter count; FL-1: Fluorescence intensity.
[b] Par: a parental cell without MDR gene.
[c] MDR: a parental cell transfected with MDR gene.

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